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INVESTIGATIVE REPORT

A Human Surrogate Model of Itch Utilizing the TRPA1 Agonist Trans-cinnamaldehyde

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The thermoreceptive transient receptor potential ankyrin 1 (TRPA1) is important in the transmission of itch, and its agonist trans-cinnamaldehyde has occasionally been reported to be a pruritogen in humans. However, no studies have accurately quantified the capabilities of trans-cinnamaldehyde to induce itch and related dysesthetic sensations. The present study examined alterations in somatosensory and vasomotor parameters in response to topical trans-cinnamaldehyde 5% and vehicle (ethanol) in 24 healthy subjects. During the study the following parameters were recorded: itch area and intensity, hyperknesis, alloknesis, neurogenic flare, skin blood flow and temperature. Trans-cinnamaldehyde evoked moderate itch sensation, flare, hyperknesis and alloknesis ($p < 0.001$). Blood flow and skin temperature were elevated in the area of trans-cinnamaldehyde application ($p < 0.001$). Significant positive correlations were found between blood flow and skin temperature, itch area and blood flow, and itch area and skin temperature. Topical trans-cinnamaldehyde proved feasible as a human itch model with applicability in studying itch mechanisms or anti-pruritic drug profiling. ***Key words: trans-cinnamaldehyde; TRPA1; itch; hyperknesis; alloknesis; experimental model.***

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Itch (pruritus) is an unpleasant sensation that evokes an urge to scratch (1). Chronic itch is a major problem that significantly disturbs patients' quality of life and poses an economic burden to societies. Treatment of chronic itch is a significant challenge (2). Itch is an extremely common symptom in many dermatological diseases (e.g. atopic dermatitis and urticaria), but it also occurs in a number of systemic disorders (e.g. chronic renal failure and cholestasis) (3).

Pruritus and pain are distinct sensations that share several common mediators and receptors (4, 5). Histamine is one of the most studied mediators of itch. Antihistamines have been used for decades for alleviating itch (6). However, chronic itch is often resistant to treatment with antihistamines. Studies have identified novel mediators and signalling pathways (histamine-independent) involved in the pathogenesis of itch (7–10), and novel antipruritics are being developed to target these components (11, 12). The thermoreceptive transient receptor potential ankyrin 1 (TRPA1) is one of the novel proposed targets for itch, while it has also been described as a potential receptor in pain signalling (13, 14). Animal models of pharmacological or genetic modulation of TRPA1 demonstrated that TRPA1 is essential for both acute histamine-independent itch (15) and chronic itch (16). Moreover, application of a TRPA1-specific antagonist, HC-030031, reverses experimentally-evoked itch in mice (17, 18). These observations support the dual contribution of TRPA1 in both itch and pain processing (18).

The aim of the present study is to assess the itch-inducing properties of TRPA1 activity by application of trans-cinnamaldehyde to healthy human skin, recording itch, itch-related psychophysical responses and vasomotor alterations.

TRPA1 is activated by several substances, including allyl isothiocyanate, gingerol, eugenol, and cinnamaldehyde (19). This ion-channel is expressed not only on sensory neurones, but also on keratinocytes (20). Human studies applying trans-cinnamaldehyde have so far investigated other roles of TRPA1, such as thermal and mechanical hyperalgesia, pain and neurogenic inflammation, but have not assessed itch beyond simply observing that subjects frequently report itch upon topical application (21, 22). The effect of cinnamaldehyde is concentration-dependent and the substance may cause a burning sensation, skin irritation, or heat hyperalgesia, especially at higher concentrations, in both rats (23) and humans (21). The multifaceted response to cinnamaldehyde might also be due to concentration-dependent effects on other TRP channels, e.g. TRPV3 and TRPM8, as described by Macpherson et al. (24).

Namer et al. (21) used 10% cinnamaldehyde and L-menthol to investigate the activation of TRPA1 and TRPM8 in humans, and coincidentally found that cinnamaldehyde also evoked a sensation of itch. Similarly, Olsen et al. (22) described that 5 out of 10 subjects reported a sensation of itch when cinnamaldehyde 10% was applied on the skin. Hence, the present study investigated whether topical trans-cinnamaldehyde

could be established as a human experimental surrogate model of itch. We proposed that topical application of trans-cinnamaldehyde would induce itch and related dysesthetic sensations, such as alloknesis and/or hyperknesis, detectable through a battery of quantitative sensory tests (QST). Since sex differences in relation to pruritus are marginally studied (25, 26), both males and females were included in the present study to investigate potential differences based on sex in response to trans-cinnamaldehyde.

MATERIALS AND METHODS (See Appendix S1[1])

RESULTS

No adverse event or wheal response was detected or reported in any participant. No significant age difference was found between included males and females (Mann-Whitney rank sum test, $p = 0.239$). Since sex-related differences were only found in skin temperature at baseline, all results and graphs with the exception of the temporal itch intensity profile, represent pooled data from the 21 included subjects.

Itch intensity and perceived itch area following trans-cinnamaldehyde

The majority of the study participants (21 of 24) reported moderate intensity of itch rated on an ordinary VAS scale (0–10) following the application of trans-cinnamaldehyde (non-responders: $n = 3$). No subjects reported itch following vehicle application. The mean peak itch intensity was 5.18 ± 0.32 (VAS 0–10) and occurred 7 min after the application of trans-cinnamaldehyde, while the average itch intensity was 3.26 ± 0.46 (Fig. 1a). The subjects were instructed to report any sensation occurring along with itch (e.g. warmth, burning, or stinging sensation) during trans-cinnamaldehyde exposure. Eight volunteers reported an innocuous warm or non-painful burning sensation together with itch and 3 described a painful burning sensation. The rest of the volunteers ($n = 13$) only reported a pure sensation of itch. The trans-cinnamaldehyde-evoked itch area drawn on the arm-charts did not show any significant differences ($p = 0.122$) between females (20.71 ± 2.35 cm^2) and males (16.54 ± 1.23 cm^2) and no notable pattern in terms of proximal-to-distal or medial-to-lateral dispersion. An insignificant trend towards better abilities to accurately localize itch on dominant arms was noted. The detailed temporal itch profile for prolonged trans-cinnamaldehyde exposure conducted in a subgroup of 4 healthy volunteers showed itch latency and peak features resembling those of the main study cohort, and in prolonged exposure the sensation of itch had subsided completely in all 4 subjects at 33 min after a slow decline from 2 (VAS 0–10) at 14 min.

Flare, skin blood flow and temperature

The application of trans-cinnamaldehyde evoked a clear vasomotor response reflected on a distinct flare area of 18.06 ± 4.59 cm^2 and significantly enhanced the superficial skin blood flow (Fig. 2a and c) compared with both vehicle and baseline measurements ($p < 0.001$), while no redness was visible following the vehicle. No sex-related differences were observed in relation to neurogenic flare areas following the application of trans-cinnamaldehyde ($p = 0.665$). The vehicle did not affect skin blood flow ($p = 0.215$).

Skin temperature also showed an increase after application of trans-cinnamaldehyde compared with vehicle ($p < 0.0001$). Vehicle did not affect skin temperature ($p = 0.317$) (Fig. 2b). Based on thermography images males generally had a higher mean baseline skin temperature than females ($p < 0.001$). However, no sex differences were found in response to the application of trans-cinnamaldehyde when assessing absolute values. When normalization to the baseline skin temperature was performed and the relative change in skin temperature was assessed between the sexes, females exhibited significantly larger increases in skin temperature than males ($p = 0.0336$), indicating that the lower baseline temperatures are compensable during neurogenic inflammation. No significant differences were found in superficial

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2103

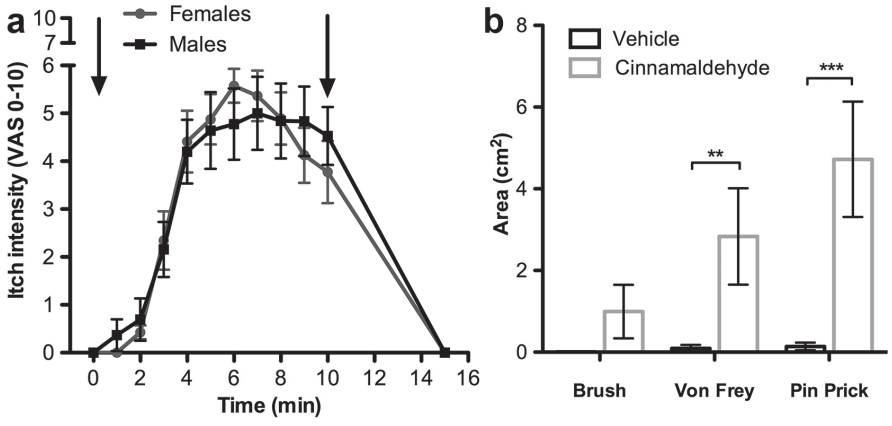

Fig. 1. (a) Temporal profile of 10 min trans-cinnamaldehyde exposure ($n = 21$). Black arrows denote the start of administration and the removal of the cinnamaldehyde patch at 10 min. (b) Areas of alloknesis (von Frey and brush) and hyperknesis (pinprick) in cm^2 following administration of trans-cinnamaldehyde and vehicle ($n = 21$). (**$p < 0.01$, ***$p < 0.001$). Vehicle evoked no response to the brush strokes.

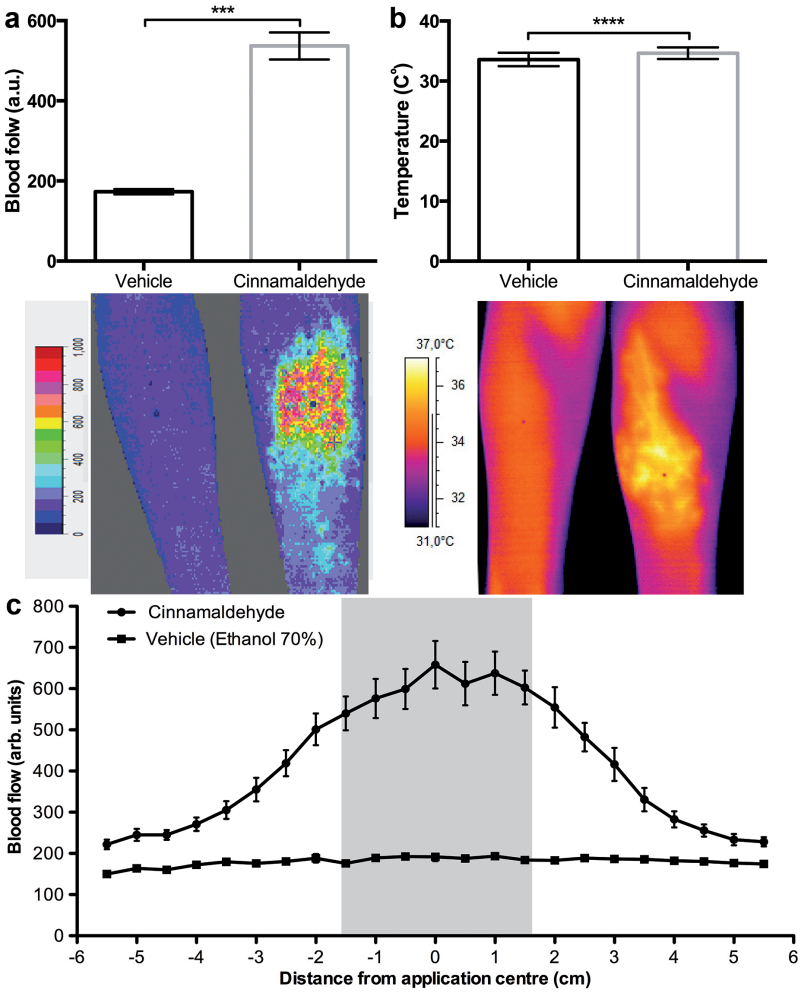

Fig. 2. (a) Skin blood flow (a.u) in a 5 × 5 cm region of interest (ROI) area surrounding the application area. Trans-cinnamaldehyde enhanced the skin blood flow compared with vehicle (***$p < 0.001$). Typical laser speckle contrast images are shown below. (b) Skin temperature (°C) in the same application areas. Trans-cinnamaldehyde enhanced the skin temperature compared with vehicle (****$p < 0.0001$). Typical thermographic pictures are illustrated below. (c) Longitudinal dispersion of neurogenic inflammation. Cinnamaldehyde caused a significant spatial spreading of neurogenic inflammation beyond the 3 × 3 cm application area, while the vehicle did not alter superficial blood perfusion. Grey represents the area of application.

blood flow and skin temperature between the means of the 3 × 3 cm region of interest area (ROI) and the 5 × 5 cm ROI area in response to trans-cinnamaldehyde, indicating a significant axon-reflex flare extending from the application area.

Sensitivity to mechanical stimulation

The mean mechanical pain threshold (MDT) at baseline was 27.3 ± 2.3, while the mean MDT was 5.3 ± 0.13. Following the application of trans-cinnamaldehyde, but not the vehicle, significant areas of both hyperknesis and alloknesis developed. The results are shown in Fig. 1b. No sex differences were detected in any of the mechanical response parameters. Following trans-cinnamaldehyde and vehicle application, the area of hyperknesis was mapped. The area of hyperknesis was detected in the trans-cinnamaldehyde-treated arm ($p = 0.001$ compared with the vehicle). All participants developed a small area of alloknesis to von Frey following the application of trans-cinnamaldehyde ($p = 0.002$ compared with the vehicle). Participants developed an area of secondary dynamic alloknesis in response to stimulation by light brush following the application of trans-cinnamaldehyde ($p = 0.017$ compared with vehicle).

Correlations

Positive correlations were found between blood flow and skin temperature ($p = 0.0256$), itch area and blood flow ($p = 0.0264$), and itch area and skin temperature ($p = 0.004$). No other significant positive or negative correlations were found between any other measured parameters, including, for example, between mechanical detection threshold and alloknesis.

DISCUSSION

This study represents the first systematic investigation of the topical application of 5% trans-cinnamaldehyde as an inducer of itch and itch-related dysesthesias in humans. We propose that trans-cinnamaldehyde could be useful as a human experimental surrogate model of itch. A distinct flare area, together with areas of alloknesis and hyperknesis, were detected in addition to a moderate evoked itch. The model generally showed no sex difference in response to the trans-cinnamaldehyde. This model might be applicable for studying TRPA1-facilitated itch in humans and can also be utilized in the testing of known or novel anti-pruritic drugs; however, it needs further investigation for validity and reproducibility and it is not exempt from limitations, including potential single observer bias, a relatively small sample size and limited dose-response data. Moreover, a few subjects ($n = 3/24$) did not report any sensation of itch in response to trans-cinnamaldehyde. The cause of this specific non-responsiveness is unknown, but TRPA1 is known to be subject to genetic variability, e.g. variant rs11988795 G.A, with resultant phenotypical differences that affect pain thresholds (32).

Trans-cinnamaldehyde-induced itch

Findings have highlighted a complex processing of itch signalling from periphery to the central nervous system with a high diversity in mediators and receptors invol-

ved (1). Several members of the TRP channels family, including TRPV1, TRPV3 and TRPA1, contribute both to pain and itch signalling in sensory neurones (14, 15, 17, 33, 34). TRP channels are not only expressed on sensory neurones, but also on cutaneous non-neuronal cells (e.g. keratinocytes) (20, 35), which makes it possible for an itch cross-talk between skin cells and sensory neurones. Basic and pre-clinical studies have revealed that histamine-dependent itch is mediated via $TRPV1^+$ mechano-insensitive C-fibres (33), while histamine-independent itch is probably conveyed via $TRPA1^+$ polymodal C-fibres (16, 17). Both of these channels have dual role (e.g. pain and itch transductions) and can be activated by endogenous and exogenous stimuli. In addition, cross-reaction (e.g. desensitization) is not uncommon between TRP channels (36). TRPA1 is also required as a downstream mediator for itch signalling by agonists such as chloroquine, bovine adrenal medulla 8-22 peptide (BAM8-22) and hydrogen peroxide (16, 17).

Trans-cinnamaldehyde is one of several exogenous TRPA1 agonists and it has been applied to human skin (e.g. forearm) and oral mucosa in different concentrations, ranging from 0.1% to 10% (37, 38). In the current study a median concentration of 5% was applied and a moderate level of perceived itch was found in the study population (mean peak itch intensity: 5.18 on $VAS_{0–10}$). Some subjects ($n = 8/24$) also reported a warm or non-painful burning sensation accompanying the itch. In line with our observations, Namer et al. (21), who also applied cinnamaldehyde on the volar forearm of healthy volunteers, but at a higher concentration (10%), reported burning pain in all subjects, but accompanying itch sensation in only 6 out of 10 subjects (60%). Based on this observation and other previous reports (24), it is possible that the effect of cinnamaldehyde is concentration-dependent and the ratio between the sensation of itch and burning pain might be based on the applied concentration. It is established that punctate delivery of capsaicin causes itch comparable with histamine or cowhage spicules, while injected capsaicin primarily elicits burning pain (39, 40). Similarly, Keele & Armstrong (41) showed, as early as 1964, that application of histamine at higher concentrations causes pain, but not itch. Differentiation or transition of perceived sensation as itch or pain, is likely a consequence of the degree to which additional nociceptive C-fibres are activated and could also explain why higher concentrations of cinnamaldehyde primarily cause burning pain, while lower concentrations mostly cause itch.

Burning sensation following cinnamaldehyde, concomitant with itch or alone has also been linked to the co-expression of TRPA1 on a subpopulation of TRPV1-positive neurones (15, 21). It is known that TRPV1 is not activated by cinnamaldehyde concentrations of up to 2 mM (24), which supports the notion of a distinction between burning sensation and itch based on concentration. Interestingly, cinnamaldehyde (0.5–5 mM) specifically activates the warmth/heat-sensing channel TRPV3 (24), which is also one of the targets identified for potential treatment of itch (34, 42). Moreover, it has been demonstrated that cinnamaldehyde inhibits TRPM8 activation, a receptor to convey a cooling sensation known to alleviate itch (2, 43). Although cinnamaldehyde has been found to evoke TRPA1-mediated itch (44), no human experimental studies have assessed cinnamaldehyde in relation to itch. Further investigation of the pruritic effect of trans-cinnamaldehyde in human skin should involve determining TRP-involvement, e.g. by pre-treatment with specific TRPV1-antagonists, such as SB705498 or 4-*tert*-butylcyclohexanol. Moreover, assessment of cross-sensitization or de-sensitization of TRP channels following the application of cinnamaldehyde alone, or in combination with, for example, menthol or capsaicin, would help enhance our understanding of the effect of cinnamaldehyde and whether it desensitizes epidermal nerve fibres in a manner analogous to that of capsaicin, and thus has potential therapeutic value.

Trans-cinnamaldehyde-induced vasomotor responses

Following application of trans-cinnamaldehyde, a flare area was detectable beyond the application site in all volunteers, which was absent in the vehicle-treated arm. These findings are in line with findings by Namer et al. (21), who also showed a cinnamaldehyde-evoked axon-reflex-flare (21). Flare is probably due to antidromic activation of the far branches of CMi-fibres that leads to release of vasoactive substances, such as calcitonin-gene related peptide (CGRP), within the peripheral tissues (45, 46). There is also a possibility of activation of mast cells and other non-neuronal cells, e.g. keratinocytes (20), that also express TRPA1 and respond to cinnamaldehyde. However, this is unlikely to be the main mechanism behind the observed flare, since release of histamine is usually accompanied by a wheal reaction (47), which was not observed in the present study. Significant flare is an uncommon finding in non-histaminergic models of itch, but a normal finding in histamine-dependent itch, typically characterized by both wheal and flare reactions. A warranted elucidation of the mechanistic basis of this model should test whether the induced itch and vasomotor reaction responses are neurogenically maintained (i.e. by lidocaine use) and/or partly refractory to antihistamines; hence, elucidating whether the model partially relies on activation of histaminergic C-fibres.

Trans-cinnamaldehyde-induced alloknesis and hyperknesis

This study is the first to show the development of areas of alloknesis and hyperknesis on human skin following application of trans-cinnamaldehyde. Areas of alloknesis and hyperknesis to mechanical stimuli have previously been detected in both histamine-dependent

and histamine-independent models (such as cowhage and punctate capsaicin), typically found to be significantly larger than the modest areas reported herein (28, 39, 48). The phenomenon has also been reported in animals, where light touch evoked scratching behaviour (49). In addition, alloknesis is frequently seen in patients with chronic itch (50). Mechanistically, development of alloknesis and hyperknesis probably relies on both peripheral and central sensitization. Peripheral sensitization results from increased excitability of the primary itch-sensing afferents (9). This theory, although not yet confirmed in humans, has been investigated in animal models, where direct evidence was found that, in the dry-skin mice model, neurones in the dorsal root ganglion responded markedly higher to SLIGRL-NH2 (a protease-activated receptor (PAR)-2 agonist) and serotonin (5-HT), but not to histamine (51). In terms of central adaptation, a prolonged or intense itch stimulus is proposed to cause increased responsiveness of the spinal neurones conveying itch to the brain, whereby activation of low-threshold mechanoreceptors and primary itch-receptive afferents can generate spinal signals of itch and increased sensation of itch (respectively) (49). However, it is unlikely that spinal mechanisms are dominant in the modest alloknesis and hyperknesis observed in the present study, since the areas generally do not extend beyond the area of application, a feature that normally signifies involvement of the central components.

Sex-related differences

Although significant sex differences are well documented in relation to pain processing, very limited data is available on acute and chronic itch (26, 52). In the present study, none of the cinnamaldehyde-evoked responses, except the relative change in skin temperature, were sex-dependent. This was related to the detection of higher skin temperatures in males compared with females at baseline, which is probably a consequence of males tending to have a higher basal metabolic rate and less subcutaneous fat (53). An insignificant tendency towards higher skin blood flow responsiveness among females was observed. Whether these observations would show up as a significant sex-related response should be assessed in a larger study population, in which parameters such as age, ethnicity and skin pigmentation could also be accounted for. Although being very limited evidence, it was noted that 2/12 males reported a burning sensation, whereas 6/12 female participants reported a burning sensation. This is in line with a study by Hartman et al. (26), wherein it was elucidated that females accentuated the burning component induced by pruritic stimulation (by punctate histamine) more frequently than their male counterparts. Moreover, the results are in line with the general consensus that females are more sensitive to pain stimulus and thus it is likely that a potential painful component of any itch model will manifest more in females (54, 55).

In summary, topical application of 5% trans-cinnamaldehyde proved feasible as a surrogate human model of itch, also encompassing itch-related dysesthetic states of alloknesis and hyperknesis, albeit to a lesser extent than is observed following the application of histamine or cowhage spicules. Trans-cinnamaldehyde was well-tolerated and showed no sex-dependency in relation to itch intensity, area of alloknesis, and hyperknesis. This model can assist in further understanding of the contribution of TRP channels to itch and pain at a human level, or serve as a suitable model to screen novel human anti-pruritic agents directed at, for example, TRPA1 or upstream target candidates.

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